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10-mers using a single amino acid overlap have been prepared from the three immunogenic domains of HIV-1 Pol which correlate best with reverse transcriptase activity (amino acids 144-191, 214-335, 511-536). These 205 overlapping decapeptides were incorporated onto pins using techology developed by Cambridge Research Biochemicals, and utilized in ELISA assays with serum samples from HIV+ individuals at various clinical stages of HIV disease. Several discrete epitopes have been identified which correlate not only with capacity to block the catalytic activity of HIV RT, but also correspond to regions identified as inhibitory using murine anti-Pol monoclonal antibodies.

Anti-Pol IgGs have been investigated for HIV-1 neutralization and enhancement in vitro. Direct viral ADE

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phenomenon was confirmed, but the level of such activity, in these specimens as well as others we'd examined, did not appear to have clinical significance.

Modified anti-sense oligodeoxynucleosides directed against gag-pol as well as tat and the NFkB enhancer sequences of HIV-1 were evaluated for the ability to block induction of latent virus. The gag-pol oligomers were ineffective, but good activity was found with others.

In the process of generating human EBV+ cell lines capable of secreting anti-Pol monoclonal antibodies, we attempted to directly infect purified peripheral blood B cells from HIV seronegative donors with HIV. Dr. Haseltine's group has recently demonstrated by PCR that, in some HIV seropositive individuals, circulating B cells may harbor provirus at levels equivalent to CD4+ T cells. Not only did we demonstrate direct infection of non-immortalized B cells by HIV but certain clones expressed a transformed, malignant phenotype, with ability to clone in soft agar, grow in 1% serum, and rapidly cause tumors of Burkitt lymphoma phenotype in SCID mice. This work has implications for the pathogenisis of B cell lymphomas in AIDS patients.

Growth hormone (somatotropin) has been used as an adjuvant in animal models to enhance B and T cell reactivity against various viral vaccines. Its ability to augment proliferation of CD4+ T cells and increase cytokine secretion in vitro and in SCID/hu mice, together with its anabolic properties, is of great interest in HIV disease. We have discovered that not only is growth hormone a potent T cell stimulant but it is capable of synergizing with the anti-HIV drugs/AZT and FLT in the inhibition of HIV replication. Preliminary data on the mechanism for this specific facilitation, not seen with the dideoxynucleosides ddI and ddC, have been gathered.

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Objectives: 1. Characterization of IgG inhibitors of HIV polymerase catalytic activity with respect to HIV neutralization and enhancement, and reactivity to linear HIV Pol sequences.

2. Correlation of anti-Pol inhibitors with cytolytic T cell activity.

3. Investigation of hormonal agents capable of enhancing immune responsivity to potential peptide-based vaccines. This is an outgrowth of one specific aim of the original application, namely, exploration of ways to prolong the latent phase of HIV infection by activating negative regulatory sequences in the HIV LTR through interactions with hormone responsive elements identified in the LTR.

Approach of research: 1. Evaluation of purified IgGs from HIV seropositive individuals with divergent levels of anti-Pol IgG inhibitor for HIV neutralizing and antibody-dependent enhancement (ADE) activity. Preparation of overlapping 10 amino acid peptides from the entire region of Pol found to be correlated with IgG anti-Pol catalytic activity inhibitors in our prior work. Use of these synthetic peptides in Pep-Scan ELISA assays to map, in fine detail, linear epitopes correlating with the inhibitory activity. Correlation of such an ELISA with the more laborious enzymatic assay in terms of clinical prediction.

2. Preparation of EBV+ B cell lines from selected individuals with high levels of anti-Pol inhibitor, in an attempt to isolate monoclonal inhibitors.

3. Use of reagents prepared in No. 1 to examine cytolytic T cells prepared from HIV seropositive individuals with varying levels of anti-Pol IgG inhibitor.

4. Definition of mechanisms of peptide hormone enhancement of anti-peptide immune responsivity.

Brief summary of accomplishments to date: 1. 10-mers using a single amino acid overlap have been prepared from the three immunogenic domains of HIV-1 Pol which correlate best with reverse transcriptase activity (amino acids 144-191, 214-335, 511-536). These 205 overlapping decapeptides were incorporated onto pins using technology developed by Cambridge Research Biochemicals, and utilized in ELISA assays with serum samples from HIV+ individuals at various clinical stages of HIV disease. Several discrete epitopes have been identified which correlate not only with capacity to block the catalytic activity of HIV RT, but also correspond to regions identified as inhibitory using murine anti-Pol monoclonal antibodies.

2. Anti-Pol IgGs have been investigated for HIV-1 neutralization and enhancement in vitro. Direct viral neutralization does not appear to explain in their activity. The ADE phenomenon was confirmed, but the level of such activity, in these specimens as well as others we'd examined, did not appear to have clinical significance.

3. Modified anti-sense oligodeoxynucleosides directed against gag-pol as well as tat and the NFkB enhancer sequences of HIV-1 were evaluated for the ability to block induction of latent virus. The gag-pol

oligomers were ineffective, but good activity was found with others.

4. In the process of generating human EBV+ cell lines capable of secreting anti-Pol monoclonal antibodies, we attempted to directly infect purified peripheral blood B cells from HIV seronegative donors with HIV. Dr. Haseltine's group has recently demonstrated by PCR that, in some HIV seropositive individuals, circulating B cells may harbor provirus at levels equivalent to CD4+ T cells. Not only did we demonstrate direct infection of non-immortalized B cells by HIV but certain clones expressed a transformed, malignant phenotype, with ability to clone in soft agar, grow in 1% serum, and rapidly cause tumors of Burkitt lymphoma phenotype in SCID mice. This work has implications for the pathogenisis of B cell lymphomas in AIDS patients.

5. Growth hormone (somatotropin) has been used as an adjuvant in animal models to enhance B and T cell reactivity against various viral vaccines. Its ability to augment proliferation of CD4+ T cells and increase cytokine secretion in vitro and in SCID/hu mice, together with its anabolic properties, is of great interest in HIV disease. We have discovered that not only is growth hormone a potent T cell stimulant but it is capable of synergizing with the anti-HIV drugs/AZT and FLT in the inhibition of HIV replication. Preliminary data on the mechanism for this specific facilitation, not seen with the dideoxynucleosides ddI and ddC, have been gathered. A more comprehensive description of some of the results, together with listings of publications arising through DAMD support, is provided below.

I. Publications acknowledging support of DAMD 17-90-Z-0049 for year 1 and 2 of grant

Original articles of direct relevance to the current work:

1. Laurence J, Saunders A, Early E, Salmon JE. 1990. Human immunodeficiency virus infection of monocytes: Relationship to Fc-gamma receptors and antibody dependent viral enhancement. Immunol. 70:338-343.

2. Laurence J, Sikder SK, Kulkosky J, Miller P, Ts'o POP. 1991. Induction of chronic human immunodeficiency virus infection is blocked in vitro by a methylphosphonate oligodeoxynucleoside targeted to a U3/enhancer element. J. Virol. 65:213-219.

3. Laurence J, Astrin SM. 1991. HIV induction of malignant transformation in human B lymphocytes. Proc. Natl. Acad. Sci. USA 88:7635-7639.

4. Laurence J, Cooke H, Sikder SK. 1990. Effect of tamoxifen on regulation of chronic HIV-1 infection and HIV LTR-directed transcription Blood. 75:696-703.

5. Laurence J, Sikder SK, Jhivari S, Salmon JE. 1990. Phorbol ester-mediated induction of HIV-1 from a chronically infected promonocyte clone: Blockade by protein kinase inhibitors and relationship to tat-associated trans-activation. Biochem. Biophys. Res. Comm. 166:349-357.

6. Laurence J, Kulkosky J, Bei D, Early E, Cianciolo GJ, Snyderman R. 1990. A soluble inhibitor of T lymphocyte function induced by HIV-1 infection of CD4+ T cells: characterization of a cellular protein and its relationship to p15E. Cell. Immunol. 128:337-352.

7. Astrin SM, Laurence J. HIV-1 activates c-myc and Epstein-Barr virus in human B cells. Ann. NY Acad. Sci., in press.

8. Laurence J, Grimison B, Gonenne A. 1992. Effect of recombinant human growth hormone on acute and chronic human immunodeficiency virus infection in vitro. Blood, 79:467-472.

9. Laurence J, Grimison B, Astrin SM. A model system for regulation of chronic HIV-1 infection in B lymphocytes. Virology, in revision.

10. Laurence J, Hodtsev AS, Posnett DN. T cell antigen receptor Vβ expression modulates HIV-1 replication, implying that HIV-1 is associated with a superantigen. Nature, in revision.

11. Sikder SK, Mitra D, Laurence J. Functional roles for a glucocorticoid responsive element in Tat-induced expression of the human immunodeficiency virus long terminal repeat. Virol., submitted.

Original articles of related relevance to the current work:

12. Laurence J. 1990. Molecular interactions among herpesviruses and human immunodeficiency viruses. J. Infect. Dis. 162:338-346.

13. Laurence J. 1990. Herpesviruses as co-factors in the immunopathogenesis of AIDS. Develop. Med. Virol. 6:249-288.

14. Roberts RB, Hollinger FB, Parks WP, Rasheed S, Laurence J, Heseltine PNR, Makuch RW, Lubina JA, Johnson KM. 1990. A multicenter clinical trial of oral ribavirin in HIV-infected patients with lymphadenopathy: Virologic observations. AIDS 4:67-72.

15. Jacobs JL, Libby DM, Winters RA, Gelmont DM, Freid ED, Hartman BJ, Laurence J. 1991. A cluster of Pneumocystis carinii pneumonia in adults without predisposing illnesses. N. Engl. J. Med. 324:246-250.

16. Dusenbury L, Botvin GJ, Baker E, Laurence J. 1991. AIDS risk knowledge, attitudes and behavioral intentions among multi-ethnic adolescents. AIDS Educ. Prevent. 3:367-375.

17. Jacobs JL, Libby DM, Hartman BJ, Laurence J. 1991. Pneumocystis carinii pneumonia in adults without predisposing illnesses. N. Engl. J. Med. 325:1313-4.

Book chapters and reviews of related relevance to the current work:

18. Laurence J. 1992. Viral co-factors in the pathogenesis of HIV infection. In Wormser GP, ed. AIDS and Other Manifestations of HIV Infection, Second Edition. (Raven Press, NY), 77-83.

19. Laurence J. 1990. Cardiac and neurovascular abnormalities in HIV infection. Infect. Med., 7:9-16.

20. Laurence J. 1990. Pathophysiology of HIV infection. Curr. Opinion Infect. Dis. 3:73-79.

21. Laurence J. 1990. Novel vaccination and anti-receptor strategies against HIV. AIDS Res. Human Retrov. 6:175-181.

22. Laurence J. 1990. Immunology of HIV infection, I: Biology of the interferons. AIDS Res. Human Retrov. 6:1149-1156.

23. Laurence J. 1991. Women and AIDS: An overview and specific disease manifestations. The AIDS Reader 1:153-159.

II. Detailed description of progress related to two specific aims of grant

A. Growth hormone (somatotropin) as a model for peptide hormone-HIV interactions. Growth hormone (GH), through induction of insulin-like growth factors I and II (IGF-I,II), has a variety of effects on lymphocyte and monocyte biology. It is also a potent anabolic agent. Because of these varied properties, there is much clinical interest in GH as a potential immune modulator and therapeutic in HIV disease. Cachexia is a common problem in advanced HIV infection and predicts a poor prognosis. In one study of hospitalized AIDS patients, 62% had lost more than 10% of their premorbid weight. Such weight loss, commonly associated with anorexia, weakness, and malnutrition ("wasting syndrome"), has serious physical and psychologic consequences, including further compromise of immune status in AIDS (1). Dr. D. Kotler of Columbia University recently noted a marked similarity in clinical outcome among studies of AIDS patients, victims of the Warsaw ghetto, and inmates at Maize Prison, Northern Ireland, undergoing lethal hunger strikes. He has uncovered instances in which the level of body weight depletion, rather than its specific etiology, appeared to be the immediate cause of death in all these cases, and speculated that if this level can be evaded—through total parenteral nutrition and/or hormonal manipulations—survival might be prolonged (2). Indeed, reduction of plasma GH levels in cats with feline AIDS parallel the growth impairment of HIV infected kittens (3), and recent studies of HIV seropositive hemophiliacs revealed evidence of dysregulation of GH secretion which paralleled wasting syndromes, a defect also seen in IV drug abusers (4).

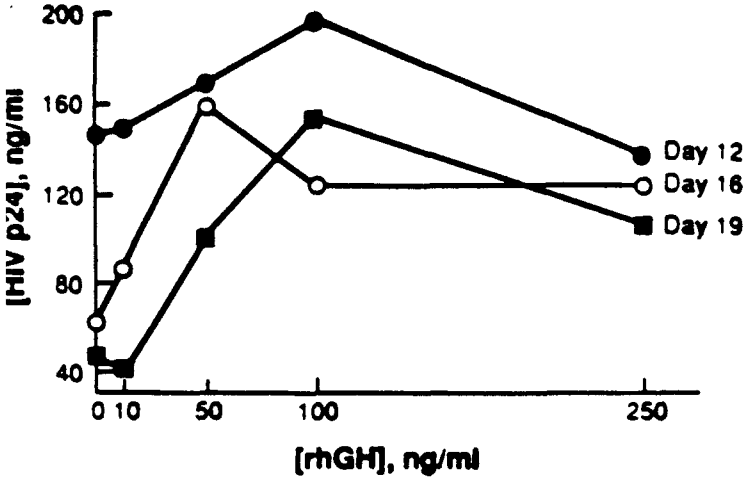
Growth hormone is thus a reasonable choice for clinical evaluation in HIV disease. It can improve nitrogen balance, support visceral protein levels, and promote lipid mobilization and protein synthesis in catabolic patients. Based on the fact that fat mobilization and oxidation are inappropriately suppressed at the expense of glucose and amino acid oxidation in infectious diseases (5), recombinant human growth hormone (rhGH) has been evaluated in a limited phase I

trial of HIV infection (6). Five milligrams of rhGH was administered subcutaneously every other day for up to 12 weeks to HIV-seropositive men with AIDS or AIDS-related complex. Prestudy weight loss was reversed ($P < .05$) in all four patients completing pharmacological dose study. This gain was associated with increases ($P < .05$) in lean body mass, in concert with a decrease in fat mass. Muscle power and endurance also improved. All four patients lost weight following termination of rhGH treatment.

There is also interest in GH as an immune modulator, as rhGH administration stimulates production and proliferation of T lymphocytes in mice, and CD4+ T cells in SCID/hu mice (7). A small pilot study to test whether GH can augment the immune systems of AIDS patients receiving anti-viral drugs is also being planned at the NCI (7). In addition, binding of rhGH and viral antigen to an insoluble matrix containing aluminum hydroxide led to markedly increased efficacy of a flavivirus (prototype, hepatitis C virus) vaccine following a single injection into mice (8). However, one concern over the use of any cytokine or growth factor in AIDS is the potential for accelerating HIV growth. Growth hormone can stimulate T cell and monocyte proliferation and cytokine production, factors that promote HIV replication in vitro. High-affinity binding sites for GH have been demonstrated on all immunocytes (9) and, as measured in the mouse, all subpopulations of immune cell—helper and cytotoxic T lymphocytes, B cells, monocytes and natural killer cells—can also secrete GH (10). In addition, sequences related to hormone responsive elements have been identified in the GH promoter, as well as the HIV-1 LTR (11); they establish the potential for upregulation by similar cellular factors. We indeed found that rhGH could enhance HIV replication and tumor necrosis factor-α (TNF-α) secretion in peripheral blood mononuclear cells (PBMC), in the absence of viral induction from chronically infected cell lines or a direct effect on HIV-1 transcription (12). The enhanced replication of HIV is consistent with rhGH-mediated stimulation of cell growth and cytokine generation. What was completely unanticipated was that not only could zidovudine (AZT) prevent the upregulation of HIV by rhGH (12), but a marked potentiation of the anti-viral effect of AZT and its fluorinated analog, FLT, was observed, in the absence of synergy with two other nucleoside analogs, ddI and ddC (See Preliminary Results). This potentiation could be tentatively related to two effects: augmentation of an AZT/FLT thymidine kinase leading to enhancement of the active HIV reverse transcriptase inhibitors, AZT-TP (triphosphate) and FLT-TP; and an increase in monophosphorylation of AZT, a form which can specifically block the RNaseH activity of HIV Pol.

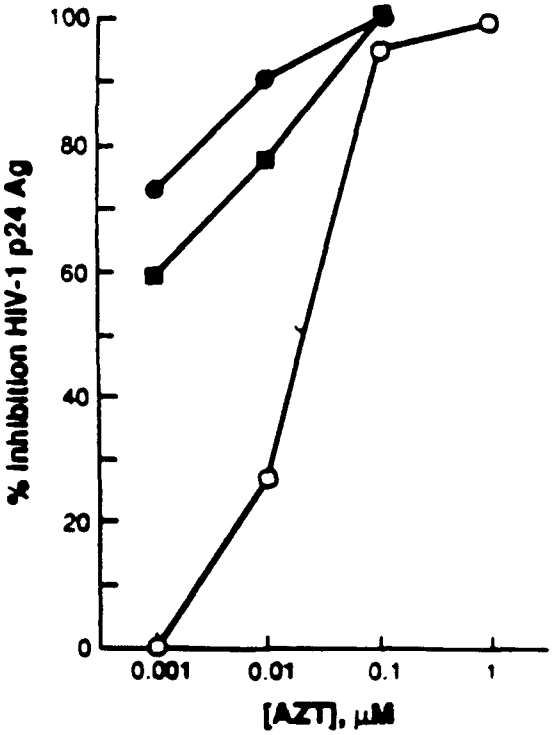
We thus exposed PHA-stimulated PBMC cultures to 1000 TCID-50 of HIV-1 TIIIB for 2 hours, followed by washing and incubation in the presence of varying concentrations of rhGH. Peak HIV-1 replication occurred in the presence of 50 ng/ml (Fig.1A). This amount and a high plateau dose (250 ng/ml), were then used along with varying concentrations (0.001-1μM) of AZT, ddC, and ddI in parallel cultures. Marked enhancement of AZT activity by rhGH was noted at all concentrations of AZT tested (Fig.1B), with an ID-50 for this laboratory strain of 0.025 μM without rhGH, and < 0.001 μM in the presence of 50 ng/ml rhGH. This occurred without alterations in cell proliferation or cell viability. In marked contrast, either no potentiation (Fig. 1C, ddI), or antagonism (Fig. 1D, ddC) were seen with the other two anti-HIV nucleoside analogs in clinical use. In terms of mechanism, Dr. Samuel Broder's lab at NCI found that GM-CSF might augment AZT's activity through induction of an AZT-specific phosphorylase, thereby raising intracellular levels of AZT-triphosphate (13). This occurred in the absence of effect, or even with some inhibition, by GM-CSF of the kinase

FIG. 1 A. Effect of rhGH on HIV replication in acutely infected PBMCs*



*p24 Gag Ag was measured in culture supernatants at the time points post-HIV exposure indicated.

FIG. 1B. Effect of rhGH + AZT on HIV replication in acutely infected PBMCs§



§Figs. 1B,C and D: open circles, no rhGH; closed circles, 50ng/ml; closed squares, 250ng/ml. All data are derived from the typical peak of HIV replication, day 14 post-inoculation.

FIG. 1C. Effect of rhGH + ddC on HIVreplication in acutely infected PBMCs§

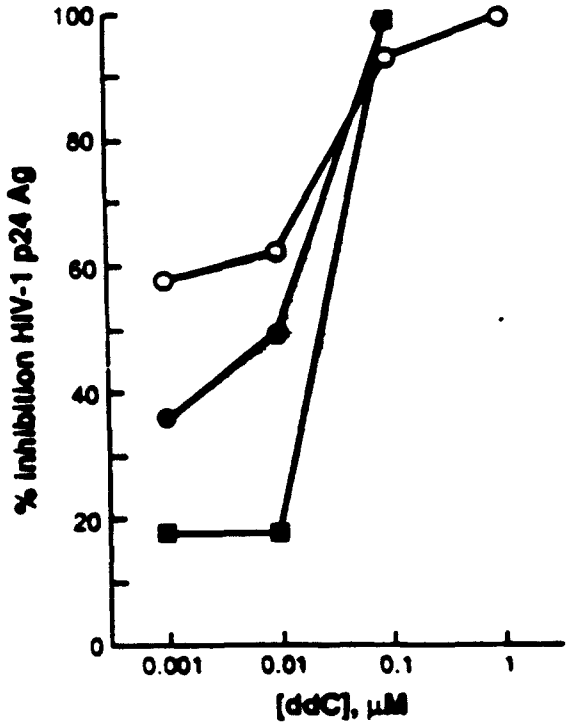
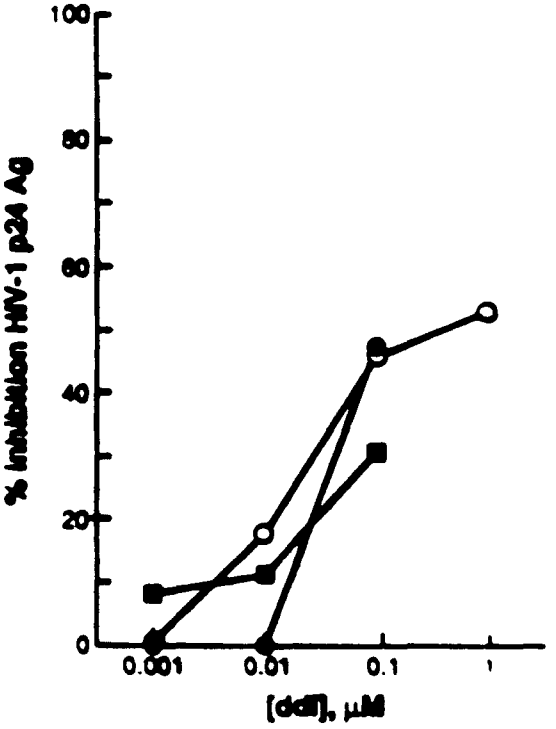


FIG. 1D. Effect of rhGH + ddI on HIV replication in acutely infected PBMCs§



necessary for triphosphorylation of ddI and ddC. We thus collaborated with Dr. Ting-Chao Chou at our neighboring institution, Memorial Sloan-Kettering Cancer Center, to measure levels of mono, di, and tri-phosphorate forms of AZT in the presence and absence of rhGH. Dr. Chou has published studies with these assays (13). As shown in Table 1, there was a 2-fold increase in triphosphorylation of AZT, and a 2.5-5 fold increase in the mono- and di-phosphorylated forms of AZT in the presence of 10 ng/ml of rhGH.

Table 1. Phosphorylation of [^{3}H]AZT Nucleotides in PBMCs in the Presence or Absence of rhGH*

Growth hormone (ng/ml)	pmole/10^6 cells of AZT		
	MP	DP	TP
0	11.06	0.21	0.26
10	26.5	0.95	0.51
50	20.2	0.61	0.33
100	21.07	0.74	0.44

*PBMCs ($2x10^6$/ml) following stimulation with PHA (5μg/ml) were incubated with 1 μM of [^{3}H]AZT in the absence and presence of various concentrations of growth hormone at 37°C for 12 hrs. Cells were washed three times and TCA extracts were analyzed with anionic exchange HPLC using PartiSphere SAX-5 column (Whatman) at 45°C with ammonium phosphate, pH 3.5. The absorbance was monitored at 270 nm.

Interestingly, this is a dose that gave the greatest stimulation of DNA synthetic response in PHA activated PBMCs, and gave relatively little enhancement of HIV-1 replication (Fig. 1A). In a very recent preliminary experiment, utilizing [^{3}H] AZT and [3H]ddI and following phosphorylated forms over the course of a typical 14 d. culture, 10 ng/ml of GH again significantly augmented mono-, di-, and tri-phosphorylation of AZT (p values < 0.01 to < 0.03) while having no effect on ddI (p = 0.4 to 0.5). These findings are encouraging in terms of clinical implications for two reasons:

1) The marked potentiation seen in vitro between AZT and rhGH in inhibition of HIV-1 replication.
2) The impact of rhGH on mono as well as tri-phosphorylation of AZT. It has recently been discovered that AZT-MP specifically blocks the RNase H activity of HIV-1 (15). Extensive screenings have been undertaken to find inhibitors of this RT associated endonuclease activity, but so far only the poorly absorbed sulfated polyanion dextran sulfate, and illimaquinone (16), appear to have activity. An agent which could increase the MP form of AZT should be of therapeutic interest in AZT resistant strains.

These data highlight the importance of following the complexities of hormonal interactions with cytokines, anti-viral drugs and other hormones, and finally to clinical trials. We have used them to support an IND, in conjunction with Bio-Technology General, Inc., to conduct a phase I rhGH trial in HIV infection.

The end points are viral activity by semi-quantitative PCR for DNA, p24 antigenemia, CD4+ T cell count, and weight change. Once completed, we would like to attempt to use rhGH as an immune potentiator of a potential Pol-based peptide vaccine as post-infection immunotherapy in HIV disease [see part B].

B. Mapping linear Pol-reactive epitopes correlating with reverse transcriptase inhibitor (RTI) activity.

Since our original description of IgG's capable of inhibiting the catalytic activity of HIV-1, -2 reverse transcriptases in the sera of asymptomatic HIV+ individuals (17), there have been numerous studies confirming the effect. Loss of this inhibitory activity has been linked to declining clinical status. It is hypothesized that certain epitopes of Pol are responsible for these inhibitory antibodies, and that such regions might serve as substrates for immunotherapy in HIV disease.

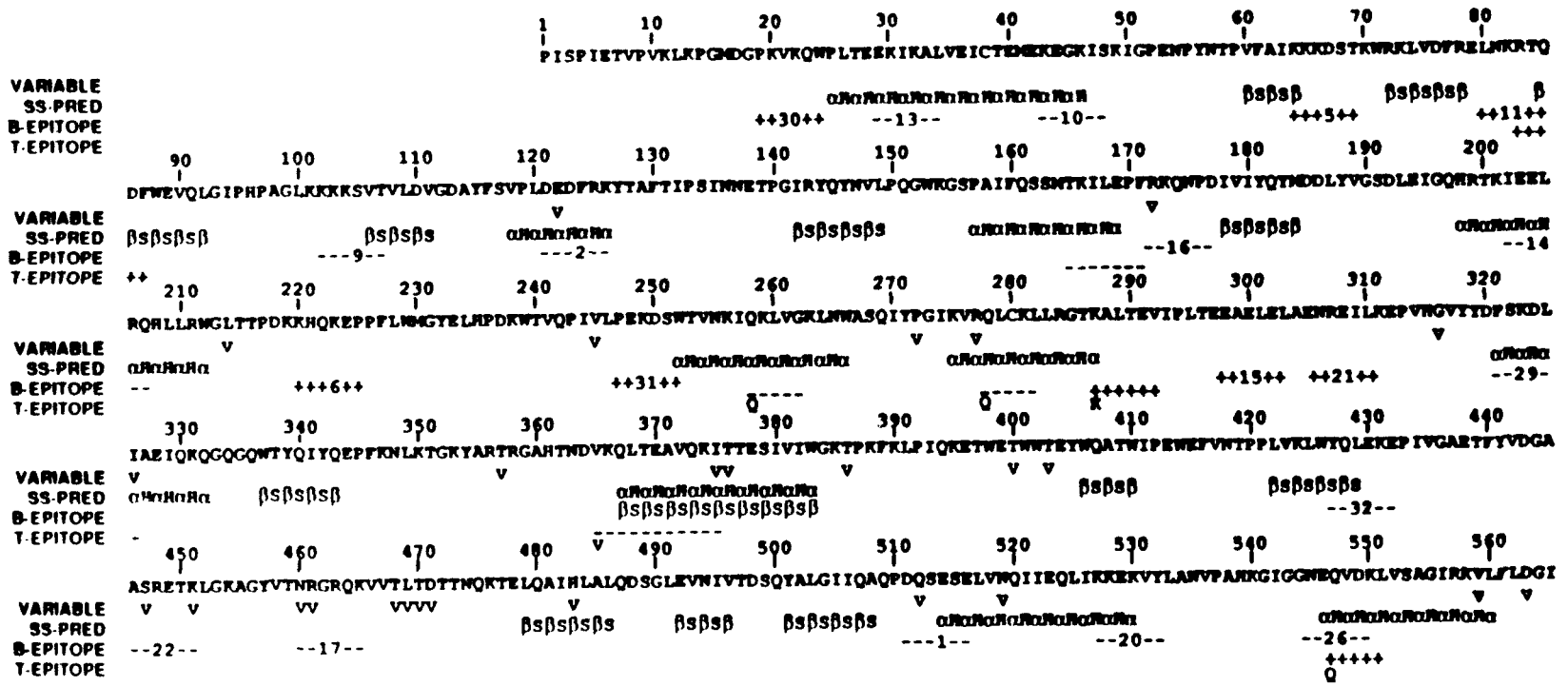
Immune-based therapies are of great interest in HIV infection, as specific immunity is the normal means of containment and eradication of viruses. Many approaches to preserving and restoring immune competence have been attempted, including cytokines and growth factors, immune modulators, receptor-directed therapy and specific immune interventions with antibodies and vaccines (18). Vaccine administration is based in part on the premise that the prolonged course of HIV infection is attributable to specific immune responses that develops to the virus shortly after exposure and that these responses are temporarily effective but eventually fail (18). This work was pioneered by Robert Redfield and colleagues using recombinant gp160 (MicroGeneSys) in patients with Walter Reed stages 1 and 2 HIV infection (19). Increased antibody to HIV was demonstrated following immunization. There was an increase in existing antibodies as well as appearance of specificities not detected during natural infection. Although the antibody induced by gp160 was derived from HIV strain IIIb, there was an increase in neutralizing activity against the MN and RF strains, and increased cellular immunity was also found in many individuals. In this progress report, we have accomplished three things. First, we have correlated humoral reactivity to six linear Pol epitopes, represented by decapeptides, with RT inhibitory (RTI) activity in selected HIV+ individuals of known RTI status as originally assessed by a functional assay. Second, we have correlated these regions of Pol with catalytic sites on Pol as mapped by others using murine monoclonal antibodies. Finally, we have initiated a screen of several hundred sera, including many longitudinal samples, from HIV+ individuals at various clinical stages of HIV disease.

Antibody-Reactive Peptide Scanning

Overlapping decapeptides of the HIV-1 polymerase protein p66 were synthesized and tested to determine the reactivity patterns of human sera. Scanning for antibody-reactive peptides (PepScan) required the synthesis of every overlapping peptide in sequence from three large immunogenic domains shown by us and others (20) to correlate with RT inhibitory activity. This sequence can be read as overlapping decapeptides, in which peptide 1 consists of residues 1-10, peptide 2 of residues 2-11 and so on. The amino acid sequence was derived from the nucleic acid sequences of the HIV-1 strain HTLV-IIIB, and for synthesis the nucleic acid sequence of the molecular clone BH10 was used.

The peptides, still coupled to solid supports, were tested against the appropriate sera by an enzyme linked immunoassay. Absorbance values (410 nm) were plotted against the position of the amino-terminal amino acid of the peptide in the total sequence. As a cut-off we considered the mean OD plus 3 SD, based upon six HIV seronegative individuals without risk factors for AIDS.

FIG. 2 MAP OF HIV-1 POLYMERASE, ILLUSTRATING REGIONS OF SEQUENCE VARIABILITY AND PREDICTED T AND B CELL STRUCTURAL EPITOPES*



*Data derived from ref. 21.

Results

Figure 2 shows the stretch of HIV-1 Pol examined, with linear B and T cell epitopes derived from secondary structure predictions by Sternberg and associates (21). Table 2 provides the amino acid sequences of the overlapping peptides studied. It is this code that is used in the numbering of the X-axis of figures 3-6.

For this study, serial samples from four homosexual males in the asymptomatic phase of HIV infection (Fig. 3A, 4A, 5A, 6A), as well as later in the course of HIV disease, when either stable clinical course (Fig. 3B, 5B, 6B), or disease progression (Fig. 4B) was noted, are mapped against Pol epitope specificity. Subsequently, 12 other HIV+ patients of various stages of disease, with known anti-RTI capacity, were similarly mapped (data not shown). From these results, five sequences were selected which appeared to correlate with high titer RTI activity. These sequences are highlighted on the Pol structure map of Fig. 7. In confirmation of the power and significance of this technique to quietly screen for such inhibitory activity, a similar map was constructed for several murine monoclonal antibodies with known capacity to inhibit HIV-1 RT, (Fig. 8) derived from the literature (22-25). There is a remarkable correlation between the epitopes identified by human sera with RTI activity and the monoclonal reagents (compare figures 7 and 8).

Future Plans

Given these data, we will now return to our bank of HIV+ sera, derived internally as well as through collaboration with Dr. Redfield, and assess reactivity to peptides shown in Fig. 7. Assuming the results continue to confirm the relevance of these epitopes to RTI, and again confirm results seen in many labs world-wide—that high RTI titers parallel stable clinical course—we would like to initiate a trial of either these peptides alone or in combination with whole recombinant HIV-1 Pol in immunotherapy of HIV disease.

We have already initiated a trial of recombinant human growth hormone as an immune adjuvant, based upon our in vitro results and those of the NCI in SCID/hu mice. Given the marked potentiation of viral vaccine responsivity in animals treated with rhGH (8), we should also like to assess the efficacy of rhGH together with vaccine immunotherapy in HIV disease. In parallel, we will continue to explore the mechanism of action of RTI in association with prolongation of the asymptomatic state of HIV infection.

TABLE 2. EPITOPE SCANNING OF HIV-1 POL

CODE	Amino Acid Sequence	Amino Acid Number	CODE	Amino Acid Sequence	Amino Acid Number
1	YQY	144 - 146	44	IYQYMDDLYV	180 - 189
2	YQYN	144 - 147	45	YQYMDDLYVG	181 - 190
3	YQYNV	144 - 148	46	QYMDDLYVGS	182 - 191
4	YQYNVL	144 - 149	47	YMDDLYVGS	183 - 191
5	YQYNVLP	144 - 150			
6	YQYNVLPQ	144 - 151	48	LTT	214 - 216
7	YQYNVLPQG	144 - 152	49	LTTP	214 - 217
8	YQYNVLPQGW	144 - 153	50	LTTPD	214 - 218
9	QYNVLPQGWK	145 - 154	51	LTTPDK	214 - 219
10	YNVLPQGWKG	146 - 155	52	LTTPDKK	214 - 220
11	NVLPQGWKGS	147 - 156	53	LTTPDKKH	214 - 221
12	VLPQGWKGSP	148 - 157	54	LTTPDKKHQ	214 - 222
13	LPQGWKGSPA	149 - 158	55	LTTPDKKHQK	214 - 223
14	PQGWKGSPAI	150 - 159	56	TIPDKKHQKE	215 - 224
15	QGWKGSPAIF	151 - 160	57	TPDKKHQKEP	216 - 225
16	GWKGSPAIFQ	152 - 161	58	PDKKHQKEPP	217 - 226
17	WKGSPAIFQS	153 - 162	59	DKKHQKEPPF	218 - 227
18	KGSPAIFQSS	154 - 163	60	KKHQKEPPFL	219 - 228
19	GSPAIFQSSM	155 - 164	61	KHQKEPPFLW	220 - 229
20	SPAIFQSSMT	156 - 165	62	HQKEPPFLWM	221 - 230
21	PAIFQSSMTK	157 - 166	63	QKEPPFLWMG	222 - 231
22	AIFQSSMTKI	158 - 167	64	KEPPFLWMGY	223 - 232
23	IFQSSMTKIL	159 - 168	65	EPPFLWMGYE	224 - 233
24	FQSSMTKILE	160 - 169	66	PPFLWMGYEL	225 - 234
25	GSSMTKILEP	161 - 170	67	PFLWMGYELH	226 - 235
26	SSMTKILEPF	162 - 171	68	FLWMGYELHP	227 - 236
27	SMTKILEPFR	163 - 172	69	LWMGYELHPD	228 - 237
28	MTKILEPFRK	164 - 173	70	WMGYELHPDK	229 - 238
29	TKILEPFRKG	165 - 174	71	MGYELHPDKW	230 - 239
30	KILEPFRKQN	166 - 175	72	GYELHPDKWT	231 - 240
31	ILEPFRKQNP	167 - 176	73	YELHPDKWTV	232 - 241
32	LEPFRKQNPD	168 - 177	74	ELHPDKWTVQ	233 - 242
33	EPFRKQNPDI	169 - 178	75	LHPDKWTVQP	234 - 243
34	PFRKQNPDIV	170 - 179	76	HPDKWTVQPI	235 - 244
35	FRKQNPDIVI	171 - 180	77	PDKWTVQPIV	236 - 245
36	RKQNPDIVIY	172 - 181	78	DKWTVQPIVL	237 - 246
37	KQNPDIVIYQ	173 - 182	79	KWTVQPIVLP	238 - 247
38	QNPDIVIYQY	174 - 183	80	WTVQPIVLPE	239 - 248
39	NPDIVIYQYM	175 - 184	81	TVQPIVLPEK	240 - 249
40	PDIVIYQYMD	176 - 185	82	VQPIVLPEKD	241 - 250
41	DIVIYQYMDD	177 - 186	83	QPIVLPEKDS	242 - 251
42	IVIYQYMDDL	178 - 187	84	PIVLPEKDSW	243 - 252
43	VIYQYMDDLY	179 - 188	85	IVLPEKDSWT	244 - 253

CODE	Amino Acid Sequence	Amino Acid Number
86	VLPEKDSWTV	245 - 254
87	LPEKDSWTVN	246 - 255
88	PEKDSWTVND	247 - 256
89	EKDSWTVNDI	248 - 257
90	KDSWTVNDIQ	249 - 258
91	DSWTVNDIQK	250 - 259
92	SWTVNDIQKL	251 - 260
93	WTVNDIQKLV	252 - 261
94	TVNDIQKLVG	253 - 262
95	VNDIQKLVGK	254 - 263
96	NDIQKLVGKL	255 - 264
97	DIQKLVGKLN	256 - 265
98	IQKLVGKLNW	257 - 266
99	QKLVGKLNWA	258 - 267
100	KLVGKLNWAS	259 - 268
101	LVGKLNWASQ	260 - 269
102	VGKLNWASQI	261 - 270
103	GKLNWASQIY	262 - 271
104	KLNWASQIYP	263 - 272
105	LNWASQIYPG	264 - 273
106	NWASQIYPGI	265 - 274
107	WASQIYPGIK	266 - 275
108	ASQIYPGIKV	267 - 276
109	SQIYPGIKVR	268 - 277
110	QIYPGIKVRQ	269 - 278
111	IYPGIKVRQL	270 - 279
112	YPGIKVRQLC	271 - 280
113	PGIKVRQLCK	272 - 281
114	GIKVRQLCKL	273 - 282
115	IKVRQLCKLL	274 - 283
116	KVRQLCKLLR	275 - 284
117	VRQLCKLLRG	276 - 285
118	RQLCKLLRGT	277 - 286
119	QLCKLLRGTK	278 - 287
120	LCKLLRGTKA	279 - 288
121	CKLLRGTKAL	280 - 289
122	KLLRGTKALT	281 - 290
123	LLRGTKALTE	282 - 291
124	LRGTKALTEV	283 - 292
125	RGTKALTEVI	284 - 293
126	GTKALTEVIP	285 - 294
127	TKALTEVIPL	286 - 295
128	KALTEVIPLT	287 - 296
129	ALTEVIPLTE	288 - 297
130	LTEVIPLTEE	289 - 298
131	TEVIPLTEEA	290 - 299
132	EVIPLTEEAE	291 - 300
133	VIPLTEEAEL	292 - 301
134	IPLTEEAELE	293 - 302
135	PLTEEAELEL	294 - 303
136	LTEEAELELA	295 - 304
137	TEEAELELAE	296 - 305
138	EEAELELAEN	297 - 306
139	EAELELAENR	298 - 307
140	AELELAENRE	299 - 308
141	ELELAENREI	300 - 309
142	LELAENREIL	301 - 310
143	ELAENREILK	302 - 311
144	LAENREILKE	303 - 312
145	AENREILKEP	304 - 313
146	ENREILKEPV	305 - 314
147	NREILKEPVH	306 - 315
148	REILKEPVHG	307 - 316
149	EILKEPVHGV	308 - 317
150	ILKEPVHGVY	309 - 318
151	LKEPVHGVYY	310 - 319
152	KEPVHGVYYD	311 - 320
153	EPVHGVYYDP	312 - 321
154	PVHGVYYDPS	313 - 322
155	VHGVYYDPSK	314 - 323
156	HGVYYDPSKD	315 - 324
157	GVYYDPSKDL	316 - 325
158	VYYDPSKDLI	317 - 326
159	YYDPSKDLIA	318 - 327
160	YDPSKDLIAE	319 - 328
161	DPSKDLIAEI	320 - 329
162	PSKDLIAEIQ	321 - 330
163	SKDLIAEIQK	322 - 331
164	KDLIAEIQKQ	323 - 332
165	DLIAEIQKQG	324 - 333
166	LIAEIQKQGQ	325 - 334
167	IAEIQKQGQG	326 - 335
168	AEIQKQGQG	327 - 335
169	EIQKQGQG	328 - 335
170	IQKQGQG	329 - 335
171	QKQGQG	330 - 335
172	KQGQG	331 - 335
173	QGQG	332 - 335
174	GQG	333 - 335
175	DQSE	511 - 514
176	DQSES	511 - 515
177	DQSESE	511 - 516
178	DQSESEL	511 - 517
179	DQSESELV	511 - 518
180	DQSESELVN	511 - 519

CODE	Amino Acid Sequence	Amino Acid Number
181	DQSESELVNQ	511 - 520
182	QSESELVNQI	512 - 521
183	SESELVNQII	513 - 522
184	ESELVNQIIE	514 - 523
185	SELVNQIIEQ	515 - 524
186	ELVNQIIEQL	516 - 525
187	LVNQIIEQLI	517 - 526
188	VNQIIEQLIK	518 - 527
189	NQIIEQLIKK	519 - 528
190	QIIEQLIKKE	520 - 529
191	IIEQLIKKEK	521 - 530
192	IEQLIKKEKV	522 - 531
193	EQLIKKEKVY	523 - 532
194	QLIKKEKVYL	524 - 533
195	LIKKEKVYLA	525 - 534
196	IKKEKVYLAW	526 - 535
197	KKEKVYLAWV	527 - 536
198	KEKVYLAWV	528 - 536
199	EKVYLAWV	529 - 536
200	KVYLAWV	530 - 536
201	VYLAWV	531 - 536
202	YLAWV	532 - 536
203	LAWV	533 - 536
204	AWV	534 - 536
205	WV	535 - 536

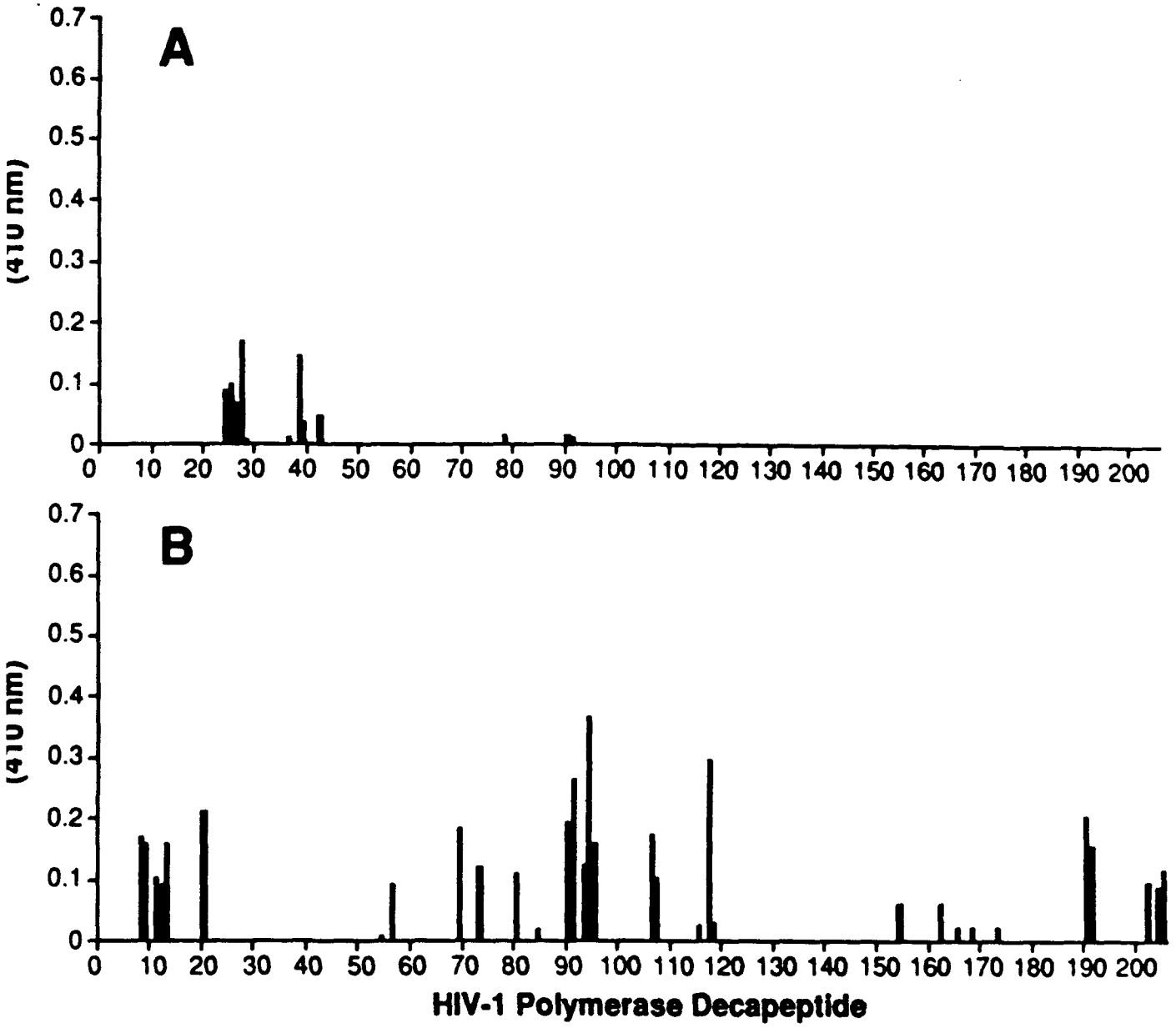


FIG. 3. PepScan Epitope map of HIV-1 Pol with Pt. 1 sera. Map A was derived using a serum sample obtained within 2 months after an acute mononucleosis-like illness. This 32 y.o. homosxual male was HIV seronegative at that time, but seroconverted within the next four months. He remained asymptomatic for almost 10 years, with moderate levels of RTI activity (50% RT inhibitory activity at a serum IgG concentration of 0.1ug/reaction mix. The map of Fig. 3B was obtained 4 years after presumptive HIV infection.

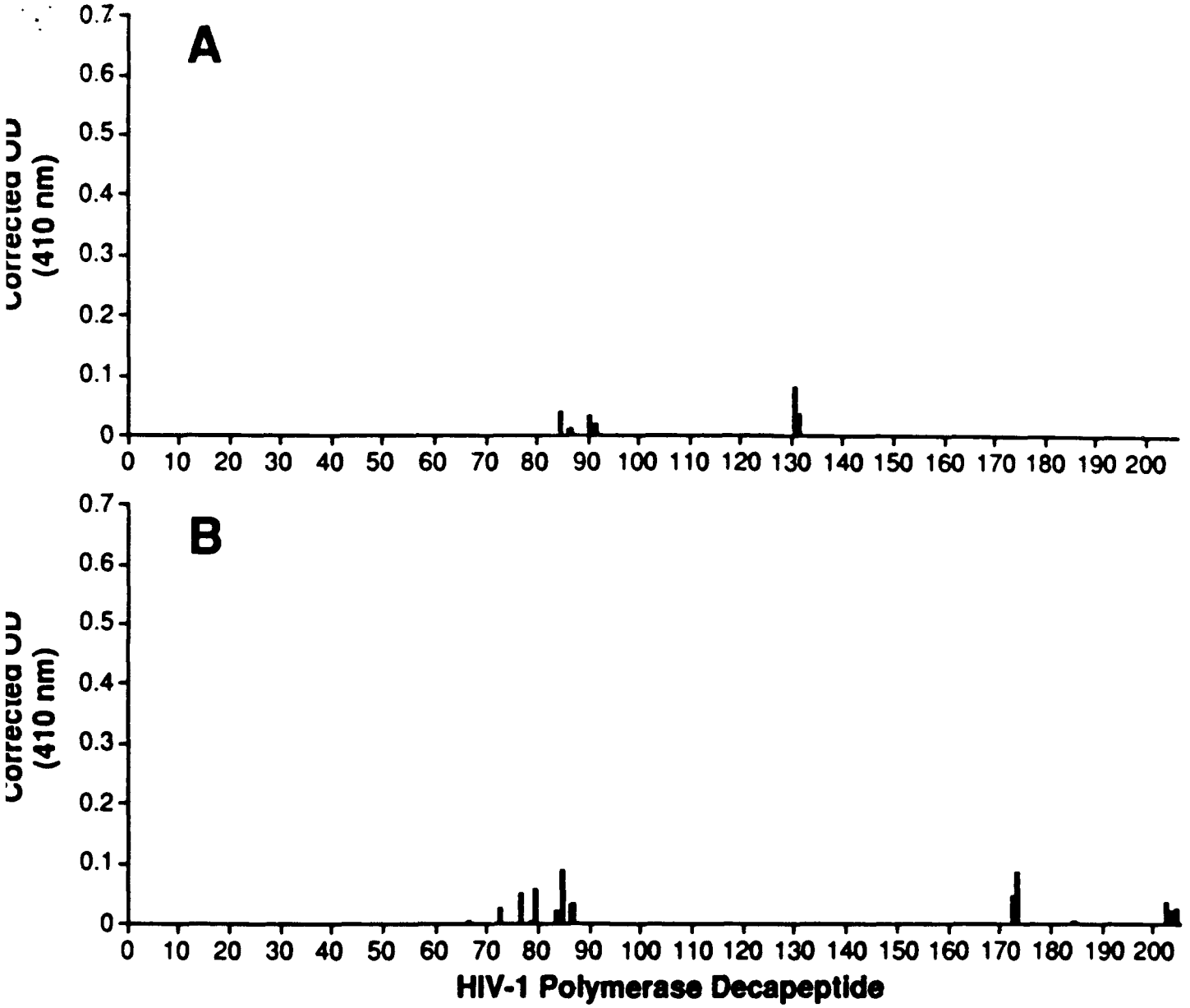


FIG. 4. PepScan Epitope map of HIV-1 Pol with Pt. 2 sera. Map A was derived using a sample obtained during the asymptomatic phase of illness, with a CD4 count of approximately 400/mm^3 and a CD4:CD8 ratio of 0.9. This individual had a rapidly progressive clinical course, with development of ARC within 15 months after presentation (Map b), and AIDS some 16 months thereafter. This individual expired 4 years after obtaining Map A, with no RTI activity identifiable at any point in his clinical course. He never received an anti-HIVmedication.

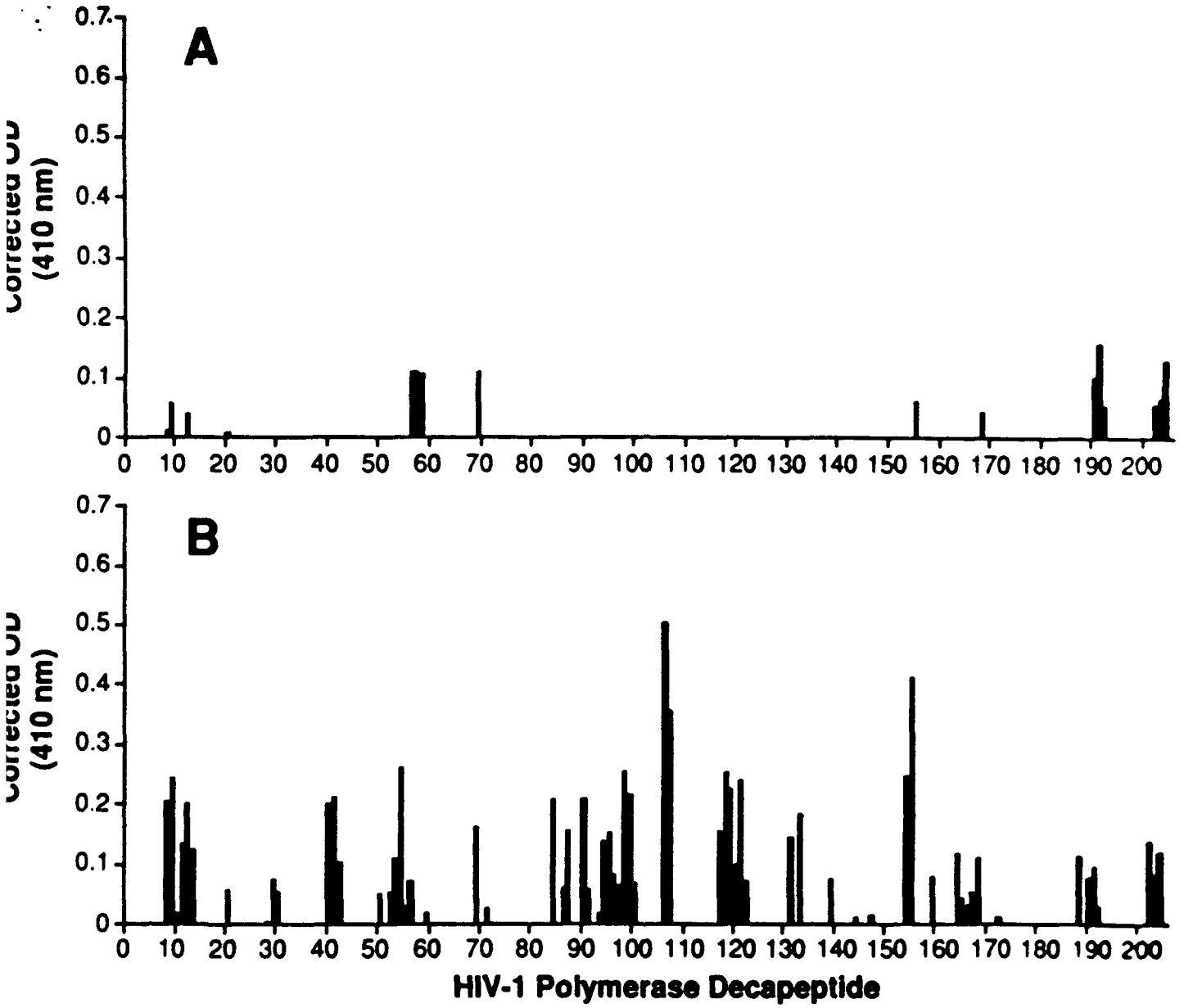


FIG. 5. PepScan Epitope map of HIV-1 Pol with Pt. 3 sera. This 35 y.o. homosexual male presented with lymphadenopathy and a CD4 count of >600/mm³ when Map A was prepared. At this time he had no RTI activity, but in serum specimens obtained biannually over the next 8 years, development of high titer activity (≥ 50% RT inhibition at a serum IgG concentration of 0.01-0.1/reaction mix) was noted. This individual has remained asymptomatic, with CD4 counts ≥350/mm³, electing to remain off all anti-HIV medications until this year. Map B was obtained four years after Map A.

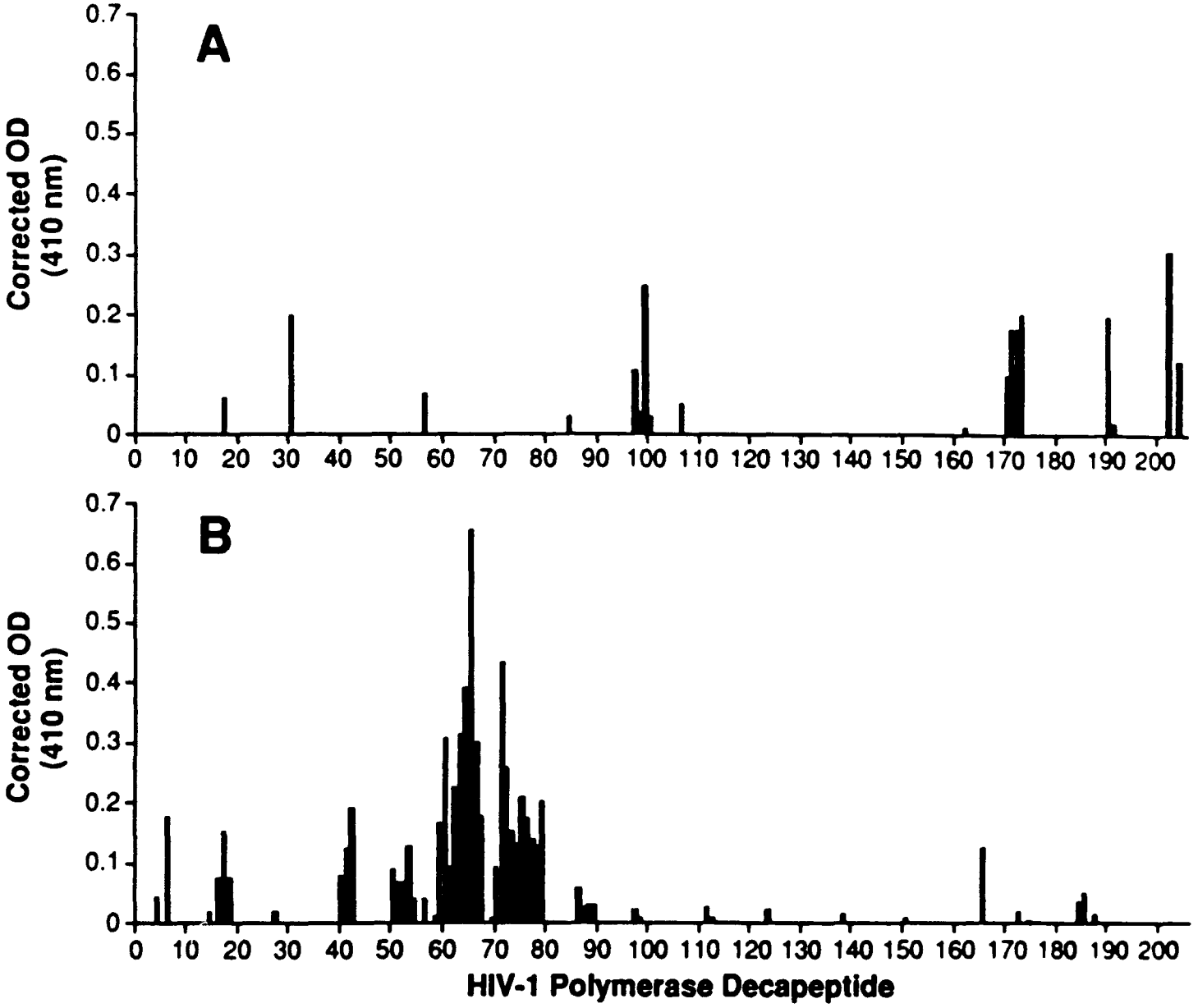
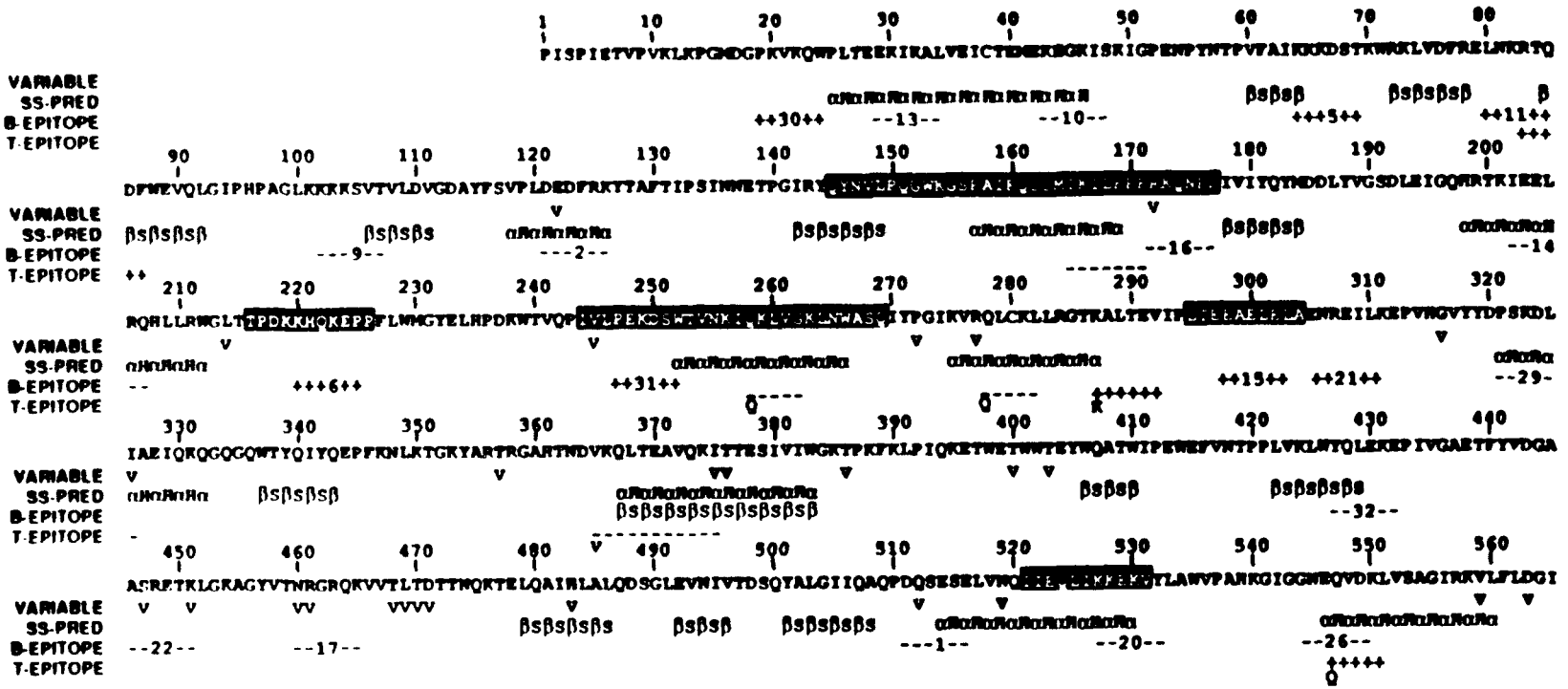


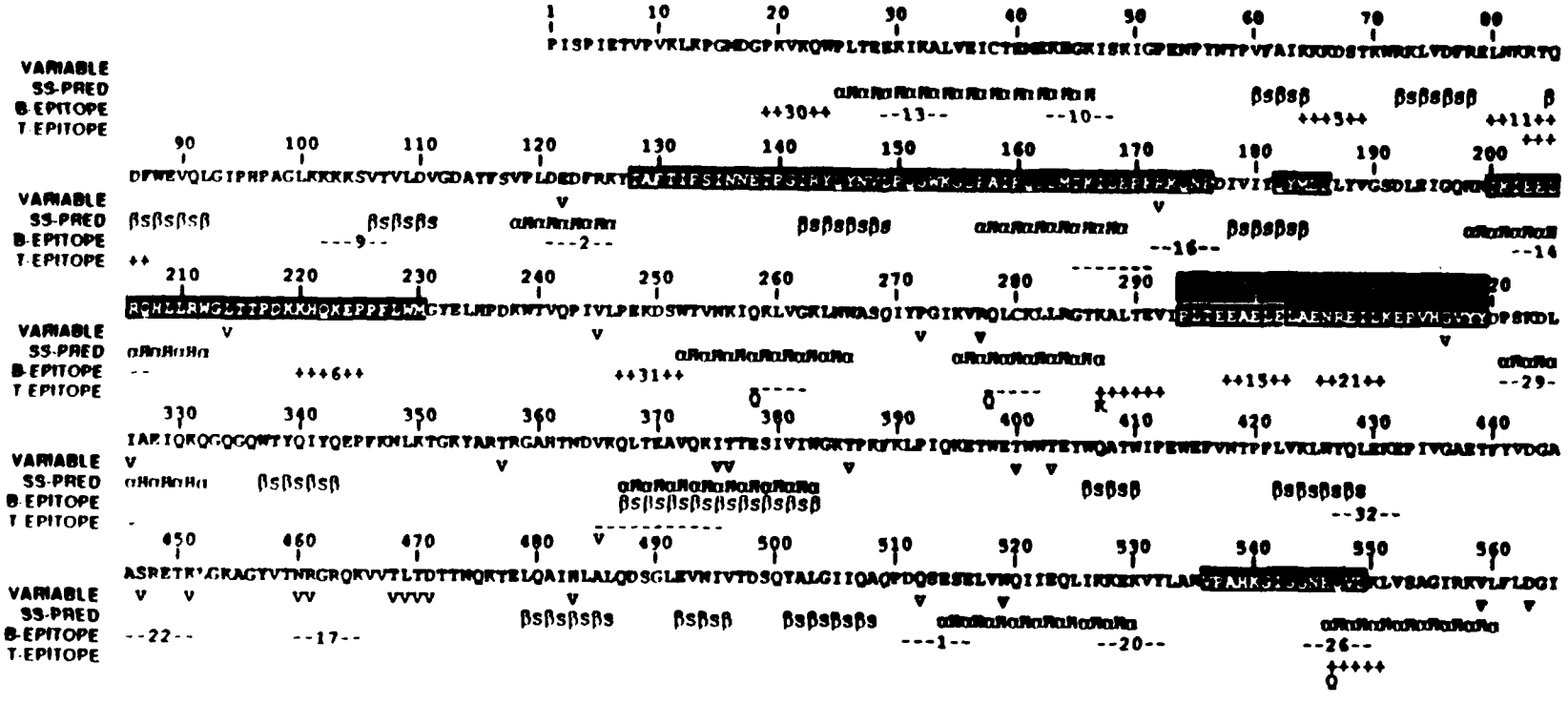
FIG. 6. PepScan Epitope map of HIV-1 Pol with Pt. 4 sera. This 45 y.o. asymptomatic homosexual male donated serum at points two years apart, when RTI activity was non-detectable (Map A), or moderate (Map B).

FIG. 7. CORRELATION OF HUMAN HIV-1+ SERA OF KNOWN REVERSE TRANSCRIPTASE INHIBITION (RTI) ACTIVITY WITH PEPSCAN REACTIVITIES DETERMINED WITH OVERLAPPING DECAPEPTIDES*



*Data correlated from 16 HIV-1+ patients (represented by serial samples from Pts. 1-4 (shown in Figs. 3-6) and 12 additional patients, as described in the text. Note that through a preliminary screen, Pepscanning was limited to residues from amino acids 144-536.

FIG. 8. EPITOPE MAP OF MURINE MONOCLONAL ANTIBODIES INHIBITORY TO THE CATALYTIC ACTIVITY OF HIV-1 REVERSE TRANSCRIPTASE*



* Data for structure predictions from ref. 21. Data for monoclonal antibody epitopes assembled from ref. 22-25.

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